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# A comparison of biochemical and paper chromatographic methods for the identification of group D streptococci from Cheddar cheese

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Summary. Forty-four strains of tributyrolytic group D streptococci isolated from Cheddar cheese were identified by physiological, biochemical and serological tests. They were also independently grouped using two-way paper chromatography in which patterns of ninhydrin positive spots were developed from 10% acetic acid extracts of the bacteria. The groupings obtained by the chromatographic and conventional techniques were then compared. Strains of *Str. durans* were well differentiated by chromatography from the other species of group D streptococci examined, but with the remainder less clear differentiation was obtained.

Chromatographic patterns of the acetic acid extracts of bacteria can be used to differentiate between species and possibly strains within species (Mattick, Cheeseman, Berridge & Bottazzi, 1956; Berridge, Cheeseman, Mattick, Bottazzi & Sharpe 1957). Studies of the casei-plantarum group of lactobacilli (Cheeseman, Berridge, Mattick, Bottazzi & Sharpe, 1957), the micrococci (Gregory & Mabbitt, 1957), the heterofermentative lactobacilli (Cheeseman, Silva & Sharpe, 1959; Cheeseman & Berridge, 1959) and a collection of lactobacilli isolated from Cheddar cheese (Cheeseman, 1960) have also been made. In general, encouraging results have been obtained, although it has been suggested that chromatography is more able to assist than supplant biochemical and serological classification.

In a survey of the types of tributyrolytic organisms present in Cheddar cheese, a large number of streptococci were isolated, of which representatives were identified by normal biochemical, physiological and serological methods as species belonging to serological group D. The first forty-four of the conventionally identified strains were then submitted to acid extraction and chromatographed by the methods used in the earlier investigations mentioned above. The results of the groupings from both methods are compared here.

## MATERIALS AND METHODS

The bacterial strains examined were isolated from nine Canadian Cheddar cheese (designated Ce, Cg, Ch, Ci, Cj, Ck, Cl, Cm and Co) and one English Cheddar cheese (Eg). The Canadian cheese were normal first grade cheese available on the United

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Kingdom market, and were 10–18 weeks old when examined. These cheese were made in seven different Ontario factories, from raw milk. The English cheese was 13 weeks old, and was made from heat-treated milk.

The cheese were sampled, and the samples emulsified and diluted by methods based on those used by Naylor & Sharpe (1958) and Robertson (1960*a*).

Bacteria capable of hydrolysing tributyrin were isolated by plating dilutions of the emulsion in the simple tributyrin agar used by Stadhouders & Mulder (1957) and a more complex medium of the following composition: peptone (Evans), 1 %; beef extract (Lemco), 0·3 %; Yeastrel, 0·3 %; NaCl, 0·5 %; Bactotryptone, 1 %; agar (Oxoid No. 3), 2 %; after adjustment to pH 6 with lactic acid, tributyrin (1 %) was incorporated by homogenizing at 90 °C to give a fine dispersion.

After incubation of the plates for 5 days at 30 °C well-isolated tributyrolytic colonies were picked from each medium into yeast dextrose litmus milk (YDLM).

The YDLM cultures were examined microscopically on coagulation or not later than 6 days after picking. Several representatives of each of the morphological types present were submitted to further tests. Those cultures which appeared to be streptococci were first tested for time to acidify, to coagulate and to cause a two-third reduction in YDLM, and for their ability to grow at 45 °C in glucose lactose yeast phosphate broth (GLYPB) (Robertson, 1960*b*). Those streptococci which grew at 45 °C were maintained in YDLM plus chalk until submitted to the following additional tests for identifying members of serological group D, broadly following the scheme of Shattock (1955). For purposes outside the scope of this paper the range of tests was rather greater than she listed. Gram staining and tests for catalase production were made with organisms grown on glucose lactose yeast phosphate agar (GLYPA). Ability to grow at 15 °C and in the presence of 4, 6, 7 and 8 % NaCl was tested in GLYPB. Tolerance of 60 °C for 30 min was tested by placing a 1 % inocula of a 24 h 1% dextrose Lemco broth (DLB) cultures into tubes of DLB immediately before holding in a water-bath at 60 ± 0·1 °C for 30 min after the temperature in a control tube reached 59·5 °C. The tubes were cooled rapidly, incubated for 2 days and examined for growth (cf. Shattock, 1949). Sensitivity to potassium tellurite (1:2500) was tested on yeast dextrose agar. This differential medium has been used by Sharpe & Shattock (private communication). Ability to hydrolyse aesculin was assessed by adding a solution of ferric ammonium chloride (10 %, w/v) to a 14-day-old culture in broth of the following composition: glucose, 0·2 %; peptone (Oxoid), 0·8 %; disodium hydrogen phosphate (anhyd.), 0·5 %; beef extract (Lemco), 1·0 %; yeast extract (Oxoid), 0·2 %; Tween 80, 0·1 %; aesculin, 0·2 %. Tests for haemolytic activity were made by streaking on nutrient agar containing 5 % horse blood (Hannay & Newland, 1950). The medium and methods of Wheater (1955) were followed for the fermentation of carbohydrates. Twenty-two of the strains (some of each species) were tested for group D antigens using the method of Shattock (1949) for concentrating the antigen.

Those strains of tributyrolytic organisms which were identified as group D streptococci were then examined chromatographically by one of us (T.I.S.) without previous knowledge of their species identification.

*Chromatographic methods*

Strains were inoculated into yeast dextrose broth (YDB) and several transfers were made until growth was vigorous. A curve showing the growth of each culture at 37 °C was then constructed by noting changes in the optical density (O.D.) at various times, using a Hilger Spekker absorptiometer. Typical growth curves are illustrated in Fig. 1. All the cultures except Ck 57 were fully grown after 6 h. It has been shown

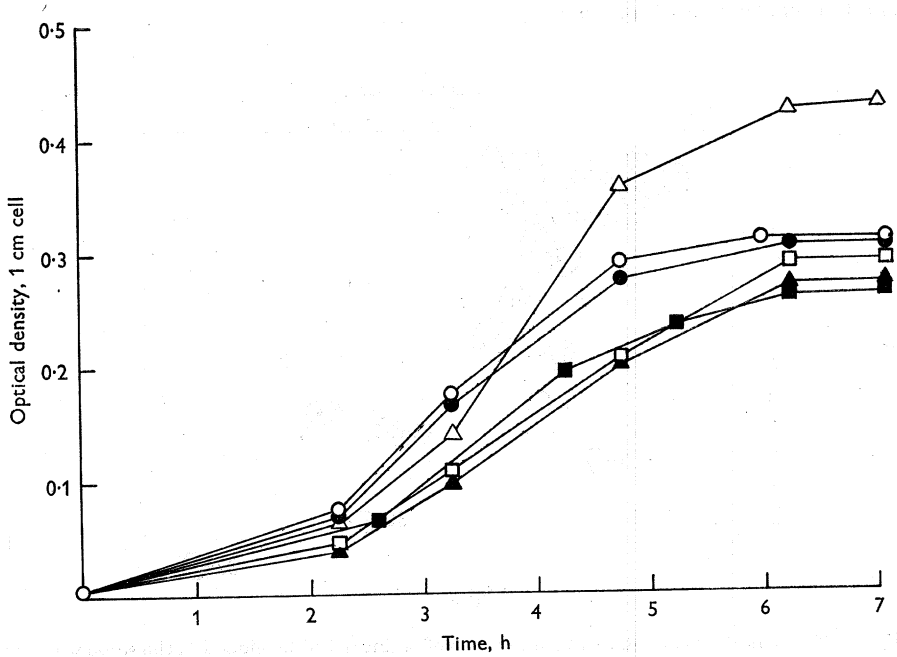


Fig. 1. Typical growth curves obtained with the streptococci in YDB.
○ Cf 51; ● Cm 51; △ Ck 54; ▲ Ck 58; □ Ck 60; ■ Cf 54.

(Berridge *et al.* 1957) that the best time for harvesting the cells was at the end of the logarithmic phase of growth or just at the beginning of the stationary phase and from our growth curves, it was decided to harvest the organisms after 6 h. In each case an inoculum of 0·4 ml from an actively growing culture was added to 20 ml of YDB, and after 6 h growth the O.D. was determined (1 cm light path) and a quantity equivalent to 10 ml of 0·3 O.D. was centrifuged, washed with water and extracted with 10 % (w/v) acetic acid (Mattick *et al.* 1956) using an extraction period of 30 min at 37 °C. After extraction the cells were centrifuged and the supernatant extract transferred to a small specimen tube.

*Paper chromatography*

The extract was spotted on Whatman No. 1 filter-paper (18¼ in. × 22½ in.) and the two-way chromatogram developed and sprayed by the method of Levy & Chung (1953). Those spots occupying positions of known amino acids (Fig. 2) were named and both known and unknown spots were numbered according to the scheme adopted

by Cheeseman *et al.* (1957). With most strains duplicate or triplicate extracts were prepared each from different subcultures grown at intervals of some weeks.

The intensity of chromatographic spots was assessed by eye and scored numerically; the maximum score (6) corresponded to the intensity of 100 $\mu$g of glutamic acid applied to 2 cm$^2$ of Whatman No. 1 filter-paper. A decrease of one unit indicated a halving of the concentration and any amino acid with a concentration score of less than 1 was indicated by T (trace). Finally, a table was constructed in which cultures giving the same spots were grouped together. In this paper no significance has been attached to spots scored as traces.

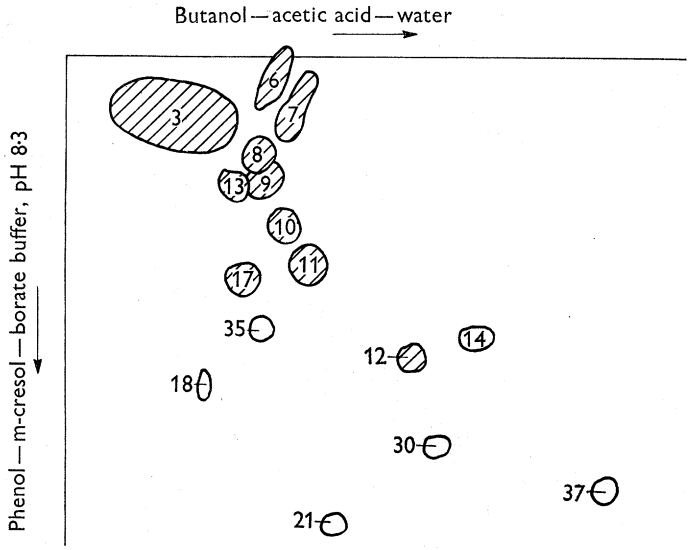


**Fig. 2.** The typical pattern shown by a mixture of amino acids developed in the same way as the bacterial extracts. The hatched spots were also found in one or more of the extracts. Spot identities: 3, lysine; 6, aspartic acid; 7, glutamic acid; 8, serine; 9, glycine; 10, threonine; 11, alanine; 12, aminobutyric acid; 13, asparagine; 14, tyrosine; 17, glutamine; 18, arginine; 21, proline; 30, methionine or valine; 35, hydroxyproline; 37, leucines.

## RESULTS

### *Chromatographic patterns and groupings*

The chromatographic patterns obtained were divided into the five main groups shown in Fig. 3. Group I contained no serine (spot 8), asparagine (spot 13) or glutamine (spot 17), group II contained serine, but no asparagine or glutamine, group III contained asparagine, but no serine or glutamine, group IV included both asparagine and serine, but no glutamine while group V contained serine, glutamine and asparagine. There were three exceptions, one similar to group II but with aminobutyric acid (spot 12), one similar to group III but with aminobutyric acid and one similar to group I but without spot 19.

Forty-three of the forty-four strains were characterized physiologically and were subdivided into five essentially homogeneous groups corresponding to the accepted subdivisions of the group D streptococci. One strain could not be classified. A few of the strains were atypical in several characteristics.

The chromatographic and biochemical data are listed in Tables 1 and 2, respectively. Comparing these Tables it may be seen that one of the three strains which fell outside the five major chromatographic groupings (Cj 57) could not be classified by conventional means and another (Co 76) was not a typical *Str. durans* for it was able to ferment mannitol and xylose and to ferment sorbitol weakly. The other strain which fell outside the chromatographic groupings (Cm 60) was a typical *Str. faecalis*

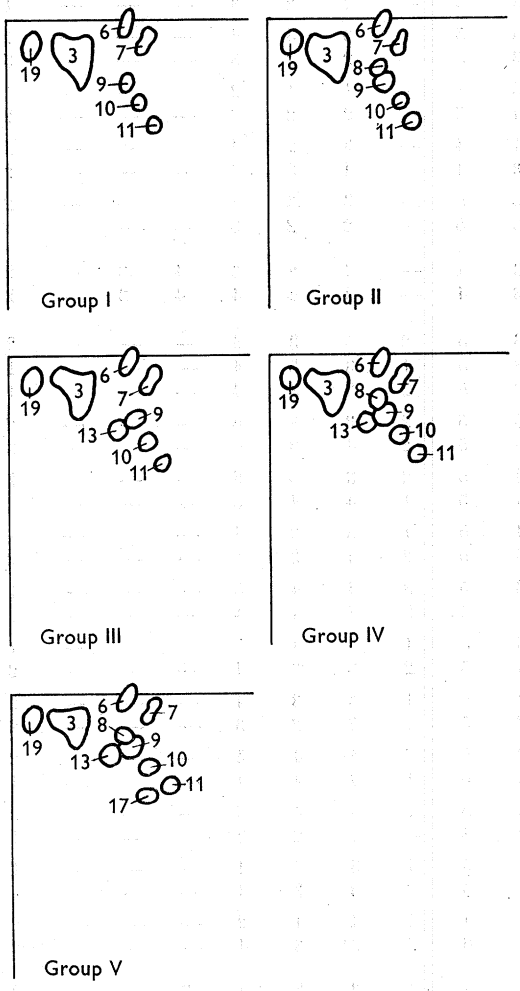


Fig. 3. The five types of chromatogram obtained with forty-one of the forty-four strains of streptococci. Spot identities: 3, lysine; 6, aspartic acid; 7, glutamic acid; 8, serine; 9, glycine; 10, threonine; 11, alanine; 13, asparagine; 17, glutamine; 19, unknown.

Table 1. *Details of the spots present and their intensities on chromatograms of extracts of streptococcal species and strains in duplicate or triplicate arranged in chromatographic groups. The identities of the organisms according to biochemical tests are also given.*

| | Spot no.* ... | 3 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 17 | 19 | Identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Spot intensity of strain | | | | | | | | | | | |
| Group I | Ci 64 | 5 | 3 | 4 | — | 2 | 2 | 3 | — | — | — | 2 | *Str. durans* |
| | | 5 | 3 | 5 | — | 2 | 2 | 3 | — | — | — | 3 | |
| | Ck 52 | 5 | 4 | 5 | — | 2 | 2 | 4 | — | — | — | 1 | *Str. durans* |
| | | 5 | 3 | 5 | — | 1 | 2 | 3 | — | — | — | 2 | |
| | Ck 54 | 5 | 4 | 5 | — | 2 | 3 | 3 | — | — | — | 3 | *Str. durans* |
| | Ck 57 | 5 | 3 | 5 | — | 2 | 3 | 4 | — | — | — | 2 | *Str. durans* |
| | | 4 | 2 | 5 | — | 2 | 2 | 3 | — | — | — | 1 | |
| | Ck 58 | 4 | 2 | 5 | — | 2 | 2 | 3 | — | — | — | 3 | *Str. durans* |
| | | 4 | 3 | 5 | — | 2 | 2 | 3 | — | — | — | 1 | |
| | Ck 60 | 5 | 3 | 5 | — | 3 | 3 | 4 | — | — | — | 1 | *Str. durans* |
| | | 5 | 3 | 5 | — | 2 | 2 | 3 | — | — | — | 3 | |
| | Cm 64 | 5 | 3 | 4 | — | 1 | 1 | 2 | — | — | — | 1 | *Str. durans* |
| | | 6 | 4 | 5 | — | 2 | 2 | 2 | — | — | — | 2 | |
| | Cm 91 | 5 | 1 | 4 | — | 3 | 2 | 4 | — | — | — | 1 | *Str. faecalis* |
| | | 6 | 3 | 4 | — | 4 | 3 | 4 | — | — | — | 2 | |
| | Eg 58 | 4 | 3 | 5 | — | 3 | 3 | 4 | — | — | — | 2 | *Str. durans* |
| | | 4 | 3 | 5 | — | 2 | 2 | 3 | — | — | — | 1 | |
| | Co 76 | 6 | 3 | 4 | — | 2 | 2 | 3 | 4 | 3 | — | 2 | *Str. durans* |
| | | 5 | 3 | 4 | — | 3 | 4 | 4 | 4 | 3 | — | 2 | |
| Group II | Ce 51 | 5 | 3 | 5 | 2 | 4 | 3 | 6 | — | — | — | 2 | *Str. faecalis* |
| | | 6 | 3 | 5 | 1 | 3 | 3 | 4 | — | — | — | 1 | |
| | | 6 | 3 | 5 | 2 | 3 | 3 | 4 | — | — | — | 1 | |
| | Ce 59 | 6 | 3 | 5 | 2 | 3 | 3 | 5 | — | — | — | 2 | *Str. faecalis* var. *zymogenes* |
| | | 6 | 3 | 5 | 2 | 4 | 3 | 4 | — | — | — | 3 | |
| | Cg 51 | 6 | 3 | 5 | 2 | 3 | 3 | 5 | — | — | — | 2 | *Str. faecalis* var. *zymogenes* |
| | | 6 | 4 | 5 | 1 | 4 | 4 | 5 | — | — | — | 1 | |
| | Ch 51 | 6 | 4 | 5 | 1 | 3 | 3 | 4 | — | — | — | 1 | *Str. faecalis* var. *liquefaciens* |
| | | 6 | 3 | 5 | 2 | 4 | 3 | 4 | — | — | — | 2 | |
| | Ch 58 | 6 | 3 | 5 | 3 | 4 | 4 | 5 | — | — | — | 1 | *Str. faecalis* |
| | | 6 | 4 | 5 | 2 | 4 | 4 | 4 | — | — | — | 2 | |
| | Ci 56 | 4 | 3 | 5 | 1 | 4 | 4 | 5 | — | — | — | 1 | *Str. faecalis* var. *zymogenes* |
| | | 6 | 4 | 5 | 2 | 3 | 3 | 4 | — | — | — | 2 | |
| | Cm 54 | 6 | 2 | 5 | 1 | 3 | 3 | 4 | — | — | — | 2 | *Str. faecalis* |
| | | 6 | 2 | 5 | 1 | 3 | 2 | 3 | — | — | — | 2 | |
| | Cm 57 | 6 | 3 | 5 | 2 | 4 | 4 | 5 | — | — | — | 2 | *Str. faecalis* |
| | | 6 | 3 | 5 | 1 | 4 | 4 | 4 | — | — | — | 1 | |
| | Co 52 | 6 | 3 | 5 | 2 | 4 | 4 | 5 | — | — | — | 2 | *Str. faecalis* var. *zymogenes* |
| | | 5 | 2 | 4 | — | 2 | 2 | 3 | — | — | — | 1 | |
| | Co 55 | 5 | 2 | 5 | 1 | 3 | 2 | 4 | — | — | — | 1 | *Str. faecalis* var. *zymogenes* |
| | | 6 | 2 | 5 | 2 | 3 | 3 | 4 | — | — | — | 1 | |
| | Eg 57 | 4 | 1 | 4 | 1 | 1 | 1 | 2 | — | — | — | 2 | *Str. faecalis* |
| | | 6 | 2 | 6 | 2 | 2 | 3 | 4 | — | — | — | 2 | |
| | Cj 57 | 6 | 3 | 4 | 1 | 3 | 3 | 3 | 3 | — | — | 2 | Unclassified |
| | | 5 | 3 | 3 | 2 | 3 | 3 | 3 | 4 | — | — | 2 | |

## Table 1 (*cont.*)

| | Spot no.* ... | 3 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 17 | 19 | Identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Spot intensity of strain | | | | | | | | | | | |
| Group III | Cf 51 | 6 | 3 | 5 | — | 4 | 4 | 5 | — | 2 | — | 2 | *Str. faecalis* |
| | | 6 | 3 | 5 | — | 4 | 4 | 5 | — | 1 | — | 2 | |
| | Ch 67 | 6 | 4 | 5 | — | 4 | 4 | 5 | — | 3 | — | 2 | *Str. faecalis* |
| | Ci 51 | 6 | 4 | 5 | — | 4 | 4 | 5 | — | 3 | — | 2 | *Str. faecalis* |
| | | 6 | 4 | 5 | — | 3 | 3 | 5 | — | 2 | — | 1 | |
| | Co 51 | 6 | 2 | 5 | — | 4 | 3 | 4 | — | 1 | — | 1 | *Str. faecalis* |
| | | 6 | 4 | 5 | — | 4 | 4 | 4 | — | 1 | — | 2 | var. *liquefaciens* |
| | Co 53 | 6 | 2 | 4 | — | 4 | 3 | 4 | — | 1 | — | 2 | *Str. faecalis* |
| | Co 80 | 5 | 3 | 5 | — | 4 | 3 | 4 | — | 3 | — | 2 | *Str. faecalis* |
| | | 3 | 2 | 3 | — | 1 | 1 | 2 | — | — | — | 1 | |
| | Cm 60 | 6 | 3 | 5 | — | 2 | 2 | 4 | — | — | — | — | *Str. faecalis* |
| | | 6 | 2 | 4 | — | 4 | 3 | 4 | — | — | — | — | |
| | | 5 | 2 | 5 | — | 3 | 3 | 4 | — | — | — | — | |
| Group IV | Ce 61 | 5 | 3 | 5 | 2 | 4 | 3 | 4 | — | 1 | — | 2 | *Str. faecium* |
| | | 6 | 4 | 5 | 1 | 3 | 2 | 4 | — | 1 | — | 2 | |
| | Cg 54 | 6 | 4 | 5 | 2 | 3 | 3 | 4 | — | 1 | — | 2 | *Str. faecalis* |
| | | 5 | 3 | 5 | 2 | 3 | 3 | 4 | — | 2 | — | 3 | |
| | Ci 52 | 5 | 3 | 5 | 2 | 3 | 3 | 5 | — | 2 | — | 1 | *Str. faecalis* |
| | | 6 | 3 | 5 | 2 | 4 | 4 | 5 | — | 3 | — | 2 | |
| | Cm 58 | 5 | 4 | 5 | 1 | 4 | 4 | 5 | — | 3 | — | 2 | *Str. faecalis* var. *zymogenes* |
| | Cm 96 | 6 | 4 | 5 | 2 | 4 | 2 | 4 | — | T | — | 2 | *Str. faecalis* |
| | | 6 | 4 | 5 | 2 | 4 | 4 | 5 | — | 3 | — | 2 | |
| | Co 66 | 6 | 4 | 5 | 3 | 3 | 3 | 4 | — | 3 | — | 2 | *Str. faecalis* |
| | | 6 | 3 | 5 | 1 | 3 | 2 | 3 | — | T | — | 2 | var. *zymogenes* |
| Group V | Ce 54 | 5 | 2 | 5 | 1 | 3 | 3 | 5 | — | 1 | — | 1 | *Str. faecalis* |
| | | 5 | 3 | 5 | 1 | 4 | 3 | 4 | — | 3 | 2 | 2 | var. *liquefaciens* |
| | Cf 54 | 6 | 4 | 5 | 2 | 4 | 4 | 5 | — | 3 | 2 | 2 | *Str. faecalis* |
| | | 6 | 2 | 5 | 2 | 2 | 2 | 4 | — | 1 | — | 1 | var. *liquefaciens* |
| | | 5 | 2 | 5 | 2 | 2 | 2 | 5 | — | 2 | 2 | 1 | |
| | Cf 65 | 6 | 4 | 5 | 2 | 4 | 4 | 5 | — | 3 | 2 | 3 | *Str. faecalis* |
| | | 5 | 3 | 5 | 1 | 3 | 4 | 5 | — | 1 | 1 | 2 | var. *liquefaciens* |
| | Ch 54 | 6 | 4 | 5 | 2 | 4 | 4 | 5 | — | 3 | 1 | 1 | *Str. faecalis* var. *liquefaciens* |
| | Ck 51 | 6 | 3 | 5 | 1 | 4 | 4 | 5 | — | 3 | 1 | 2 | *Str. faecalis* var. *liquefaciens* |
| | Cm 51 | 6 | 3 | 5 | 3 | 4 | 3 | 5 | — | 3 | 1 | 3 | *Str. faecalis* |
| | Cm 92 | 4 | 4 | 5 | 3 | 4 | 4 | 5 | — | 2 | 2 | 1 | *Str. faecalis* |
| | | 5 | 2 | 5 | 1 | 3 | 3 | 4 | — | — | — | 1 | |
| | Co 72 | 6 | 4 | 5 | 1 | 4 | 4 | 5 | — | 3 | 1 | 2 | *Str. faecalis* |
| | | 5 | 2 | 5 | T | 3 | 2 | 4 | — | 1 | T | 1 | var. *liquefaciens* |
| | Eg 51 | 6 | 4 | 5 | 1 | 4 | 4 | 5 | — | 3 | 1 | 2 | *Str. faecalis* |
| | | 6 | 4 | 5 | — | 4 | 3 | 4 | — | — | — | 2 | var. *liquefaciens* |

Spot intensity scale: —, Trace (T), 1, 2, 3, 4, 5, 6.

* See Figs. 2 and 3 for identities.

strain in its physiological and biochemical characteristics but as may be seen from Table 3 the strains of *Str. faecalis* examined were heterogeneous in their chromatographic patterns.

Table 2. *Physiological, biochemical and serological characteristics of the strains of tributyrolytic group D streptococci which were examined in detail*

| | *Str. faecalis* | | | | | | | | | | | | | | | | *Str. faecalis* var. *zymogenes* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Eg 57 | Ce 51 | Cf 51 | Cg 54 | Ch 58 | Ch 67 | Ci 51 | Ci 52 | Cm 51 | Cm 54 | Cm 57 | Cm 60 | Cm 91 | Cm 92 | Cm 96 | Co 53 | Co 80 | Ce 59 | Cg 51 | Cg 56 | Cm 58 | Co 52 | Co 55 | Co 66 |
| YDLM R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | $R^3$ | R | $R^3$ | R | R | R | R | R | R | $R^2$ |
| YDLM C | C | C | C | C | $C^2$ | $C^2$ | C | C | C | C | C | C | C | C | $C^2$ | C | $C^2$ | C | C | C | C | C | C | C |
| Fermentation | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose | − | ± | ± | − | ± | − | − | − | + | − | − | − | − | − | − | ± | − | ± | ± | − | − | ± | − | − |
| Dextrin | − | + | ± | . | + | ± | + | + | + | + | + | + | + | + | ± | + | + | + | + | + | + | + | + | ± |
| Glycerol | . | + | + | . | . | . | + | + | + | + | + | + | + | + | + | ± | ± | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | − |
| Mannitol | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Melizitose | + | + | + | + | + | + | + | + | ± | + | + | + | + | + | + | + | + | ± | − | + | + | + | + | + |
| Melibiose | − | − | − | − | ± | − | − | − | − | − | − | − | ± | − | − | − | − | − | − | − | − | − | − | − |
| Sorbitol | + | + | + | + | + | + | + | + | + | + | + | + | + | + | ± | ± | | + | + | + | + | ± | + | ± |
| Sucrose | . | + | + | . | . | . | + | . | + | + | + | + | + | + | + | + | ± | + | ± | + | + | − | + | − |
| Xylose | − | − | − | ± | − | − | − | + | + | − | − | − | − | − | − | − | − | ± | ± | ± | − | ± | − | − |
| Hydrolysis aesculin | + | ± | ± | + | + | + | − | − | + | + | + | + | + | + | + | + | + | + | + | ± | + | + | + | + |
| Liquefaction gelatine | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | + | − | − |
| Haemolysis | γ | g | g | γ | γ | γ | g | γ | g | g | g | g | g | g | g | g | g | β | β | β | β | β | β | β |
| Growth K tellurite | . | + | + | . | . | . | + | . | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Serological group | D | D | . | . | D | . | . | . | D | D | . | . | . | . | . | . | . | D | . | D | . | D | . | D |

| | *Str. faecalis* var. *liquefaciens* | | | | | | | | | *Str. faecium* | *Str. durans* | | | | | | | | | U |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Eg 51 | Ce 54 | Cf 54 | Cf 65 | Ch 51 | Ch 54 | Ck 51 | Co 51 | Co 72 | Ce 61 | Eg 58 | Ci 64 | Ck 52 | Ck 54 | Ck 57 | Ck 58 | Ck 60 | Cm 64 | Co 76 | Cj 57 |
| YDLM R | R | R | R | R | R | R | R | R | R | $R^2$ | $R^4$ | R | $R^2$ | $R^2$ | $R^2$ | $R^2$ | $R^2$ | $R^3$ | R | $R^3$ |
| YDLM C | C | C | C | C | $C^2$ | C | C | C | C | C | $C^2$ | C | C | C | C | C | C | $C^2$ | C | $C^3$ |
| Fermentation | | | | | | | | | | | | | | | | | | | | |
| Arabinose | − | ± | − | − | − | − | − | ± | − | + | − | − | − | − | − | − | − | − | − | + |
| Dextrin | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − | ± | ± | − |
| Glycerol | . | + | + | . | + | . | + | + | + | ± | . | ± | ± | . | . | ± | ± | ± | ± | + |
| Maltose | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + |
| Mannitol | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − | + | + |
| Melizitose | + | + | + | + | + | + | − | + | + | − | − | − | − | − | − | − | − | − | − | − |
| Melibiose | − | − | − | − | − | − | − | − | − | + | + | + | + | + | − | ± | + | + | − | + |
| Sorbitol | + | + | + | + | + | + | + | + | + | ± | − | − | − | − | − | − | − | − | ± | + |
| Sucrose | . | + | + | . | + | . | + | ± | + | ± | . | − | − | − | − | − | − | + | − | + |
| Xylose | + | + | − | − | + | + | − | − | − | − | + | − | − | − | − | − | − | − | + | − |
| Hydrolysis aesculin | + | + | + | − | + | + | . | + | + | − | + | + | . | . | . | . | . | + | + | + |
| Liquefaction gelatine | + | + | + | + | + | + | + | + | + | + | − | − | − | − | − | − | − | − | − | − |
| Haemolysis | g | g | g | γ | γ | g | g | g | g | g | β | g | g | g | β | β | β | g | g | β |
| Growth K tellurite | . | + | + | . | + | . | + | + | + | − | − | − | − | − | − | − | − | − | − | − |
| Serological group | . | D | D | . | . | D | . | D | . | D | D | D | D | D | . | D | . | D | D | D |

U = Unclassified. g = greening of blood near colony. + = strong positive reaction. ± = weak positive reaction, − = negative reaction, . = not tested. R = reduced, C = acid clot (R or C reactions taking more than 1 day are shown by appropriate superscripts).

All strains were catalase negative; grew at 15 and 45 °C and after heating at 60 °C for 30 min; could tolerate 8 % NaCl (Co 72 only tolerated 7 %); were able to ferment cellobiose, lactose, salicin, and trehalose but not raffinose (Cj 57 and Cm 64 were able to ferment raffinose).

Table 3. *Numbers of isolates conventionally identified in each chromatographic group*

| Chromatographic group | Conventionally defined species | | | | | |
|---|---|---|---|---|---|---|
| | *Str. faecalis* | *Str. faecalis* var. *liquefaciens* | *Str. faecalis* var. *zymogenes* | *Str. faecium* | *Str. durans* | Unclassified |
| I | 1 | — | — | — | 8 | — |
| II | 5 | 1 | 5 | — | — | — |
| III | 5 | 1 | — | — | — | — |
| IV | 3 | — | 2 | 1 | — | — |
| V | 2 | 7 | — | — | — | — |
| No group | 1 | — | — | — | 1 | 1 |

## DISCUSSION

The object of this work was to compare the techniques of paper chromatography with the normal biochemical and physiological methods of identification of group D streptococci isolated from Cheddar cheese. The streptococci examined may have been somewhat atypical as they were isolated for their tributyrolytic activity. Little is known of the tributyrolytic activity of group D streptococci, although Long & Hammer (1936, 1937) have reported tributyrolytic activity in *Str. liquefaciens* (now regarded as *Str. faecalis* var. *liquefaciens*; *Bergey's Manual*, Breed, Murray & Smith, 1957).

It appears from this limited amount of material that the technique of paper chromatography is more successful in differentiating strains having wide biochemical differences than strains which are similar biochemically. In particular the results suggest (Table 3) that *Str. durans* can be differentiated from other group D streptococci by its chromatographic pattern. The other strains, *Str. faecalis* var. *liquefaciens*, *Str. faecalis* var. *zymogenes* and *Str. faecalis* were more evenly spread over the remaining groups. However, with two of these three species the chromatographic technique enabled some differentiation to be made and the results show most of the *Str. faecalis* var. *liquefaciens* to be in group V with spots corresponding to asparagine and glutamine and most of the *Str. faecalis* var. *zymogenes* to be in group II which is without these amino acids. The distinction between these two variants of *Str. faecalis* in the conventional scheme of classification (Shattock, 1955) depends on a single criterion, the presence or absence of $\beta$-haemolysis of horse blood. The chromatographic results tend to support the distinction between liquefaciens and zymogenes. In a similar context it would be interesting to know whether *Str. faecium* is chromatographically more closely related to *Str. durans* than to *Str. faecalis*.

The spot intensities shown in Table 1 are not sufficiently different from one another (allowing for an error of $\pm 1$ in visual readings) to allow further differentiation within the defined groups.

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